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Glyphosate, pathways to modern diseases IV: cancer and related pathologies

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Glyphosate is the active ingredient in the pervasive herbicide, Roundup, and its usage, particularly in the United States, has increased dramatically in the last two decades, in step with the widespread adoption of Roundup®-Ready core crops. The World Health Organization recently labelled glyphosate as "probably carcinogenic." In this paper, we review the research literature, with the goal of evaluating the carcinogenic potential of glyphosate. Glyphosate has a large number of tumorigenic effects on biological systems, including direct damage to DNA in sensitive cells, disruption of glycine homeostasis, succinate dehydrogenase inhibition, chelation of manganese, modification to more carcinogenic molecules such as N-nitrosoglyphosate and glyoxylate, disruption of fructose metabolism, etc. Epidemiological evidence supports strong temporal correlations between glyphosate usage on crops and a multitude of cancers that are reaching epidemic proportions, including breast cancer, pancreatic cancer, kidney cancer, thyroid cancer, liver cancer, bladder cancer and myeloid leukaemia. Here, we support these correlations through an examination of Monsanto's early studies on glyphosate, and explain how the biological effects of glyphosate could induce each of these cancers. We believe that the available evidence warrants a reconsideration of the risk/benefit trade-off with respect to glyphosate usage to control weeds, and we advocate much stricter regulation of glyphosate.

Keywords: cataracts, CYP 450 enzymes, glyphosate, gut microbiome, interstitial disease, kidney cancer, non-Hodgkin's lymphoma, pancreatic cancer

1. INTRODUCTION

Glyphosate is the active ingredient in the pervasive herbicide, Roundup. Its usage on crops to control weeds in the United States and elsewhere has increased dramatically in the past two decades, driven by the increase over the same time period in the use of genetically modified (GM)[1] crops, the widespread emergence of glyphosate-resistant weeds among the GM crops (necessitating ever-higher doses to achieve the same herbicidal effect), as well as the increased adoption of glyphosate as a desiccating agent just before harvest. GM crops include corn, soy, canola (rapeseed) and sugar beet [1]. Crop desiccation by glyphosate includes application to non-GM crops such as dried peas, beans and lentils. It should be noted that the use of glyphosate for pre-harvest staging for perennial weed control is now a major crop management strategy. The increase in glyphosate usage in the United States is extremely well correlated with the concurrent increase in the incidence and/or death rate of multiple diseases, including several cancers [1]. These include thyroid cancer, liver cancer, bladder cancer, pancreatic cancer, kidney cancer and myeloid leukaemia, as shown in Table 1, reproduced from [1]. The World Health Organization (WHO) revised its assessment of glyphosate's carcinogenic potential in March 2015, relabelling it as a "probable carcinogen" [2, 3].

Table 1. Pearson's coefficients between time trends in various cancers and glyphosate applications to corn and soy crops, over the interval from 1990–2010, along with corresponding *P*-values, as determined from hospital discharge data and death data maintained by the US Centers for Disease Control (CDC). Table adapted from Swanson et al. 2014 [1].

Disease	*R*	*P*
Thyroid cancer (incidence)	0.988	$\leq 7.6 \times 10^{-9}$
Liver cancer (incidence)	0.960	$\leq 4.6 \times 10^{-8}$
Bladder cancer (deaths)	0.981	$\leq 4.7 \times 10^{-9}$
Pancreatic cancer (incidence)	0.918	$\leq 4.6 \times 10^{-7}$
Kidney cancer (incidence)	0.973	$\leq 2.0 \times 10^{-8}$
Myeloid leukaemia (deaths)	0.878	$\leq 1.5 \times 10^{-6}$

Sri Lanka's newly elected president, Maithripala Sirisena, banned glyphosate imports as one of his first acts following election. This action was based on studies by Jayasumana et al. that provided compelling evidence that glyphosate was a key factor in the chronic kidney disease that was affecting an alarming number of young

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[1] Usually called genetically engineered (GE) in the USA.

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agricultural workers in the northern region [4, 5], and was probably further motivated by the WHO reevaluation of its carcinogenic potential. Kidney disease is a risk factor for multiple cancers, with kidney dialysis being associated with increased risk of Kaposi's sarcoma by more than 50-fold, with 3- to 10-fold increased risk of kidney cancer, and 2- to 9-fold increased risk of thyroid cancer. Many other cancers also show more modest risk increases [6].

A study of rats fed GM maize and/or Roundup in their water over their entire lifespan revealed significantly increased risk of massive mammary tumours in the females, along with kidney and liver damage in the males [7]. Most of the tumours were benign, but there were three metastases (in female animals) and two Wilm's tumours found in the kidneys of males, which had to be euthanized early due to the excessive tumours, which grew to more than 25% of their body size. The exposed animals also had a shortened life span compared to the controls.

The hormone oestrogen was declared to be a human carcinogen by the National Toxicology Program in 2003 [8]. Glyphosate has been demonstrated to have oestrogenic effects at minute dosages, in *in vitro* experiments on mammary tumour cells [9]. Glyphosate was able to induce proliferation in these cells in concentrations of parts per trillion,[2] and it did so through binding affinity to the oestrogen receptor and inducing activation of the oestrogen response element (ERE). The fact that an oestrogen antagonist, ICI 182780, could inhibit glyphosate's action demonstrated rather conclusively that it was mediated through oestrogen mimicry.

Traditional concepts in toxicology are centred on Paracelsus' dictum that "the dose makes the poison", meaning that one should expect an increasing risk of toxicity as the level of exposure is increased. However, the generality of this concept has been challenged due to the realization that endocrine-disrupting chemicals (EDCs) often show a greater potential to cause cancer at very low doses than at higher doses; i.e., the relationship between dose and response is nonmonotonic, with higher doses producing a lower toxic effect than lower doses. In fact, levels of exposure well below the lowest level used in standard toxicology studies can be carcinogenic, as discussed by Vandenberg et al. [10]. These authors concluded their abstract as follows: "We illustrate that nonmonotonic responses and low-dose effects are remarkably common in studies of natural hormones and EDCs. Whether low doses of EDCs influence certain human disorders is no longer conjecture, because epidemiological studies show that environmental exposures to EDCs are associated with human diseases and disabilities. We conclude that when nonmonotonic dose-response curves occur, the effects of low doses cannot be predicted by the effects observed at high doses. Thus, fundamental changes in chemical testing and safety determination are needed to protect human health."

Glyphosate is toxic to many microbes as well as to most plants, and one likely effect of chronic low-dose oral exposure to glyphosate is a disruption of the balance among gut microbes towards an over-representation of pathogens [11]. This leads to a chronic inflammatory state in the gut, as well as an impaired gut barrier and many other sequelae. It has become increasingly apparent that chronic inflammation increases cancer risk and, in fact, many inflammatory conditions, such as Crohn's disease, hepatitis, schistosomiasis, thyroiditis, prostatitis and inflammatory bowel disease are known cancer risk factors [12].

In this paper, we review the research literature on glyphosate, with particular emphasis on evidence of carcinogenic potential, which includes glyphosate's induction of metabolic disorders, oxidative stress and DNA damage, known precursors to cancer development. We begin with a section that summarizes our own findings following perusal of large numbers of documents that were provided to one of us (Samsel) by the US Environmental Protection Agency (EPA), according to the Freedom of Information Act, which provided detailed information on Monsanto's own early experimental animal studies on glyphosate.

This section motivates and inspires subsequent sections where we seek to explain the likely mechanisms by which glyphosate might cause the tumours observed in Monsanto's studies as well as explaining the strong statistical correlations with human cancers. Following a section that provides direct evidence of DNA damage, the next four sections discuss metabolic disorders linked to glyphosate that are known to increase cancer risk, including succinate dehydrogenase inhibition, glycation damage, N-nitrosylation, and disrupted glycine homeostasis. The subsequent eight sections successively address cancer of the colon, liver, pancreas and kidney, melanoma, thyroid cancer, breast cancer, and lymphoma. In each section we provide evidence of a link to glyphosate from the research literature and propose plausible explanations for a causal link. We finally conclude with a summary of our findings.

[2] U.S. trillion, i.e. 10^{12}.

4. MONSANTO'S EARLY STUDIES

One of us (Samsel) petitioned the EPA for copies of documents originating from Monsanto, dating from the 1970s through the 1980s, which described experiments conducted by Monsanto to evaluate whether glyphosate is safe for human consumption. In this section, we provide a summary of our findings related to those documents, especially with respect to indications of kidney damage, tumorigenicity, bioaccumulation, and glyphosate metabolites.

4.1 Kidney damage

Classification of types of kidney damage, which are indicative of kidney disease, are noted below, based on information contained in Monsanto's glyphosate studies on rats and mice [13–18]. In [13], changes in the kidneys associated with chronic progressive neuropathy were noted mostly in males, but also in some female animals of both control and treated groups. There was also mineralization and mineralized debris found in the pelvic epithelium of the kidney, most often in females.

Following submission of the study, the EPA subsequently asked Monsanto for a histological re-examination of the low- and mid-dose male animals, which resulted in establishing a no observable effect level (NOEL). In response, Monsanto submitted an addendum [14] to the pathology report. The results of the addendum summarized the examination of the kidneys and found minimal tubular dilatation accompanied by interstitial fibrosis in all test groups. Statistically significant increases in tubular dilatation of the kidney were noted. A 50% increase in changes to the kidney of the low-dose group and, in the high-dose group, a fourfold increase in incidence was found compared to the control.

Interstitial renal fibrosis begins with an accumulation of extracellular matrix proteins, which is the result of inflammation and injury to the cells, which is found in every type of chronic kidney disease (CKD). Interstitial fibrosis is a progressive pathogenesis leading to end-stage renal failure [19].

The results of the 1981 study [17] further found:

1. Focal tubular hyperplasia, a hyperplasia of the tubular epithelium of the kidney caused by repeated tubular damage. It is characterized by an abnormal increase in the number of cells, which causes enlargement. Tubular epithelial hyperplasia precedes the pathogenesis of tubular dilatation in acute tubular necrosis [20].
2. Focal tubular dilation, a swelling or flattening of the renal tubule, seen as a result of an ischaemic or toxic event as in pharmaceutical, antibiotic or chemical poisoning. This leads to acute tubular necrosis, a cause of acute kidney injury and kidney failure.
3. Focal tubular nephrosis, a degenerative disease of the renal tubules of the kidney. This nephrosis is a noninflammatory nephropathy that features damage of the renal tubules [21].
4. Interstitial mononuclear cell infiltrate characteristic of inflammatory lesions, which consist of white blood cells that clear debris from an injury site.

Mineral deposits can be indicative of kidney stones, which may be calcium oxalate deposits inside the kidney, as we shall discuss more fully later in this paper.

A 1983 chronic feeding study in mice [16] found a carcinogenic response to glyphosate in both male and female mice. There was also an increased incidence of chronic interstitial nephritis in male animals. The study, lasting 18 months, involved feeding glyphosate by diet using concentrations of 1000, 5000 and 30 000 ppm. The incidence of kidney tumours in the control animals was 0/49, as was also noted in the lowest dose group. However, the mid-dose and high-dose groups produced incidences of neoplasms at 1/50 and 3/50 respectively, which caused the EPA Oncogenicity Peer Review Committee to temporarily classify glyphosate as a Class C carcinogen.

Monsanto, dissatisfied with the action, consulted another pathologist who, upon further examination, found a small tumour in the control. This was followed by the EPA using a number of pathologists to re-examine additional kidney sections from the mice to check the validity of the findings. However, their re-examination did not find any additional tumours nor confirm the tumour in the control animal. There were no tumours present in any additional sections. EPA asked for the decision to be externally refereed by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel, who found the results were statistically significant even after comparing the data to historical controls. However, the committee agreed to downgrade glyphosate to a Class D compound, arguing inadequate evidence of oncogenicity, and further sealed the study as a trade secret of Monsanto.

Non-neoplastic changes included:

1. Renal tubular neoplasms (in male mice; none found in females);
2. Chronic interstitial nephritis (in males);
3. Renal tubular epithelial basophilia and hyperplasias (decreased in males, but a dose-related increase found in females);
4. Proximal tubule epithelial cell basophilia and hypertrophy (females).

4.2 Tumorigenicity

A 26-month long-term study in rats conducted by Bio/dynamics revealed multitudes of tumours in glands and organs [13]. They occurred (from highest to lowest

incidence) in the following organs: pituitary, thyroid, thymus, mammary glands, testes, kidney, pancreas, liver and lungs. Pituitary, thyroid and thymus glands control body and immune function, and disruption can induce disease, including cancer. These glands produce many necessary hormones that control numerous biological processes. Tumorigenic growth also disrupts functionality of the glands and organs where the growth occurs. A Monsanto trade secret document [13] revealed that there were statistically significant lymphocytic hyperplasias of the thymus as well as significant C-cell thyroid tumours. Thymus lymphoid hyperplasia occurs in Graves disease and thymus hyperplasia is commonly observed with computed tomography (CT) scans of thyroid cancer patients [22], and is also associated with autoimmune disorders such as myasthenia gravis, lupus erythematosis, scleroderma and rheumatoid arthritis [23].

It should be noted that significant incidence of tumours was found during these investigations. However, to create doubt and obscure the statistical significance of inconvenient findings, which may have prevented product registration, Monsanto used experimental noise from 3, 5, 7 and even 11 unrelated study controls to effectively eliminate results, as needed. In some instances the experiments' own control showed 0% incidence of tumours, while the results for the glyphosate-treated groups were statistically significant. However, through the dishonest magic of comparing the findings to data from unrelated historical controls, they were explained away as a mystery and deemed not to be related to administration of the glyphosate.

Using these deviations effectively neutralized the inconvenient results and thus allowed the product to be brought to market. Had they not engaged in this deception, glyphosate may never have been registered for use. EPA documents show that unanimity of opinion for product registration was not reached. Not all members of the EPA glyphosate review committee approved the registration of glyphosate. There were those who dissented and signed "DO NOT CONCUR."[3]

The EC GLP document [24] notes: "Misdosing and/or cross-contamination of the test item is always a risk in animal studies. These problems are usually detected by the presence of the test item and /or its metabolites in plasma or other biological samples from control animals. It is recognized that dietary and topical studies might lead to a higher level and incidence of contamination of test item in control animals. However, contamination of biological samples from control animals has been observed also in studies using other routes of administration, e.g. gavage, intravenous, intraperitoneal, subcutaneous or inhalation. Exposure of the control animals to the test item may compromise or invalidate the study from a scientific point of view."

Thus, these unrelated historical controls were most likely corrupted studies, whether by technician error, contaminated water and /or feed, or other mistakes. This explains Monsanto's collusion with the EPA and the subsequent hiding of the data from purview.

Data tables are presented in Tables 2 through 7, without the use of experimental noise from historical controls. Only the data results of the experiment are shown.

Table 2. 1981 Bio/dynamics 26-month glyphosate feeding study [17]: interstitial cell tumours of the testes in Sprague Dawley rats.

Glyphosate dose /mg kg^{-1} day^{-1}	0	3	10	30
Terminal sacrifice	0/15 (0%)	2/26 (7.69%)	1/16 (6.25%)	4/26 (15.38%)
All animals	0/50 (0%)	3/50 (6%)	1/50 (2%)	6/50 (12%)

Table 3. 1981 Bio/dynamics 26-month glyphosate feeding study [17]. Incidence of kidney focal tubular dilatation (FTD) and focal tubuler nephrosis (FTN) in Sprague Dawley rats.

Glyphosate dose /mg kg^{-1} day^{-1}	0	3	10	30
FTD unilateral	2/10 (20%)	3/10 (30%)	2/9 (22%)	7/10 (70%)
FTD bilateral	0/10 (0%)	2/10 (20%)	1/9 (11%)	1/10 (10%)
FTN unilateral	1/10 (10%)	2/10 (20%)	1/9 (11%)	0/10 (0%)
FTN bilateral	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)

[3] The practice of introducing "experimental noise" by using data from unrelated historical controls is still in use today, but is obviously really bad laboratory practice. The European Union Good Laboratory Practice (GLP) Working Group approved a guidance document for GLP inspectors and test facilities in 2005; it is available at the European Commission (EC) GLP internet site [24]. The document discusses the responsibilities of the study director and the principles of identifying misdosing as well as corrective measures.

Table 4. 1981 Bio/dynamics 26-month glyphosate feeding study [17]: incidence of pancreatic islet cell tumours in male Sprague Dawley rats.

Glyphosate dose /mg kg^{-1} day^{-1}	0	3	10	30
Adenomas	0/50 (0%)	5/49 (10%)	2/50 (4%)	2/50 (4%)
Carcinomas	0/50 (0%)	0/49 (0%)	0/50 (0%)	1/50 (2%)
Adenomas and carcinomas	0/50 (0%)	5/49 (10%)	2/50 (4%)	3/50 (6%)
Hyperplasias	3/50 (6%)	2/49 (4%)	1/50 (2%)	0/50 (0%)

Table 5. 1990 Stout & Rueker 24 month glyphosate feeding study [15]: incidence of pancreatic islet cell tumours in male Sprague Dawley rats.

Glyphosate dose (ppm)	0	2000	8000	20 000
Adenomas	1/43 (2%)	8/45 (18%)	5/49 (10%)	7/48 (15%)
P	0.170	0.018	0.135	0.042
Carcinomas	1/43 (2%)	0/45 (0%)	0/49 (0%)	0/48 (0%)
P	0.159	0.409	0.467	0.472
Adenomas and carcinomas	2/43 (5%)	8/45 (18%)	5/49 (10%)	7/48 (15%)
P	0.241	0.052	0.275	0.108
Hyperplasia	2/43 (5%)	0/45 (0%)	3/49 (6%)	2/48 (4%)
P	0.323	0.236	0.526	0.649

Table 6. 1990 Stout & Rueker 24 month glyphosate feeding study [15]: incidence of thyroid C-cell tumours in male Sprague Dawley rats.

Glyphosate dose (ppm)	0	2000	8000	20 000
Adenomas	2/54 (4%)	4/55 (7%)	8/58 (14%)	7/58 (12%)
P	0.069	0.348	0.060	0.099
Carcinomas	0/54 (0%)	2/55(4%)	0/58 (0%)	1/58 (2%)
P	0.452	0.252	1.000	0.518
Adenomas and carcinomas	2/54 (4%)	6/55 (11%)	8/58(14%)	8/58 (14%)
P	0.077	0.141	0.060	0.060
Hyperplasia	4/54 (7%)	1/55 (2%)	5/58 (9%)	4/58 (7%)
P	0.312	0.176	0.546	0.601

Table 7. 1990 Stout and Rueker 24 month glyphosate feeding study [15]: incidence of thyroid C-cell tumours in female Sprague Dawley rats.

Glyphosate dose (ppm)	0	2000	8000	20 000
Adenomas	2/57 (4%)	2/60 (3%)	6/59(10%)	6/55 (11%)
P	0.031	0.671	0.147	0.124
Carcinomas	0/57 (0%)	0/60 (0%)	1/59 (2%)	0/55 (0%)
P	0.445	1.000	0.509	1.000
Adenomas and carcinomas	2/57 (4%)	2/60 (3%)	7/59 (12%)	6/55 (11%)
P	0.033	0.671	0.090	0.124
Hyperplasia	10/57 (18%)	5/60 (8%)	7/59 (12%)	4/55 (7%)
P	0.113	0.112	0.274	0.086

In a long-term study conducted by Monsanto between 1987 and 1989 [15], glyphosate was found to induce a statistically significant ($P<0.05$) cataractous lens formation, highest in male rats. Considerably higher doses of glyphosate, i.e., 2000, 8000 and 20 000 ppm, were administered to low-, mid- and high-dose animals respectively, as compared to long-term studies conducted on mice and rats in the early 1980s. Over the course of the study, cataract lens changes were seen in low-, mid- and high-dosed groups of both male and female rats. A second pathology

examination also found statistically significant changes (see Table 8). The pathologist concluded that there was a glyphosate-treatment related response for lens changes to the eyes. Monsanto documents also revealed an increased incidence of basophilic degeneration of the posterior subcapsular lens (fibroses) in highly dosed males.

Table 8. Incidence and occurrence of ophthalmic degenerative lens changes by glyphosate [15].

	Control	Low-dose	Mid-dose	High-dose
Male rats	2/14 (14%)	3/19 (16%)	3/17 (18%)	5/17 (29%)
All Animals	4/60 (7%)	6/60 (10%)	5/60 (8%)	8/60 (13%)

At the conclusion and termination of the experiment, further incidence of degenerative lens changes was revealed, as shown in Table 8. The ophthalmic examination yielded no noticeable changes to the control animals (0/15 or 0.0%); however, highly dosed males were significantly impacted as 5/20 (25%), as shown in Table 9. The study again noted that "the occurrence of degenerative lens changes in high dose male rats appears to have been exacerbated by (glyphosate) treatment" [15]. Unrelated historical controls were used to negate all findings.

Table 9. Data on unilateral and bilateral cataracts (all types) and Y-suture opacities, excluding "prominent Y suture", following glyphosate exposure to rats. From Stout & Rueker (1990) [15].

Sex	Group	No. Examined	No. Affected	% Affected
Male	N	15	0	0
	1	22	1	5
	2	18	3	17
	3	20	5	25
Female	N	23	0	0
	1	24	0	0
	2	17	1	6
	3	19	2	11

Stout & Ruecker [15] noted in a two year study with chronic feeding of glyphosate in rats: "Histopathological examination revealed an increase in the number of mid-dose females displaying inflammation of the stomach squamous mucosa. This was the only statistically significant occurrence of non-neoplastic lesions." Incidence of lesions of the squamous mucosa are shown in Table 10. Again, Monsanto used unrelated historical controls to negate these findings.

Table 10. Lesions of the stomach squamous mucosa in rats chronically exposed to glyphosate at three different levels (adapted from Stout and Ruecker [15].

	Controls	Low	Mid	High
Glyphosate (ppm)	0	2000	8000	20000
Males	2/58 (3.44%)	3/58 (5.17%)	5/59 (8.47%)	7/59 (11.86%)
Females	0/58 (0.00%)	3/60 (5.00%)	9/60 (15.00%)	6/59 (10.16%)

4.3 Bioaccumulation

Ridley and Mirly [25] found bioaccumulation of ^{14}C-radiolabelled glyphosate in Sprague Dawley rat tissues. Residues were present in bone, marrow, blood and glands including the thyroid, testes and ovaries, as well as major organs, including the heart, liver, lungs, kidneys, spleen and stomach. Further details are shown in Table 11. A low-dose, oral absorption (10 mg/kg body weight) of the radiolabelled xenohormone indicated highest bioaccumulations. The 1988 Monsanto study disclosed: "A significantly greater percentage of the dose remained in the organs and tissues and residual carcasses for the males than for the females. Overall recoveries for group 5 animals were 92.8% and 94.2% for males and females respectively."

The study examined seven test groups of 3 to 5 animals per sex/group that were administered a single radiolabelled dose of glyphosate. Blood, expired air, faeces and urine were collected and analysed by liquid scintillation counting

(LSC), and glyphosate with its metabolites analysed by two methods of high pressure liquid chromatography (HPLC). Three animals were used in groups sacrificed at the end of 24 hours, and 5 animals were used for all other groups, which were sacrificed at the end of the seven day study. Groups 3 and 7 received a 10 mg/kg intravenous dose, while group 4 a high oral dose (1 g/kg). Groups 1, 2, 5 and 6 each received a single oral 10 mg/kg radiolabelled dose. Group 6 animals received multiple low doses of 10 mg/kg of nonradiolabelled glyphosate for 14 days prior to administration of a single 10 mg/kg radiolabelled dose.

Oral absorption of glyphosate was 30% and 35%, with a radiological β half-life of 7.5 and 14 days in male and female animals, respectively. Bioaccumulation of glyphosate found in bone was 0.748 ppm for males and 0.462 ppm for females for group 6 animals. Group 5 animals retained 0.552 ppm and 0.313 ppm for males and females, respectively. Males also had higher levels of glyphosate in their blood. Approximately 0.27% of the orally administered dose was found in expired CO_2 of the group 1 rats sacrificed after 24 hours. Table 11 shows the mean average and percentage distribution of radioactivity in ppm that were found in tissues and organs of groups 4, 5 and 6 of the orally dosed animals.

Table 11. Distribution and bioaccumulation of ^{14}C radiolabelled glyphosate in blood, bone, glands, organs and other tissue of Sprague Dawley rats. Data obtained from Ridley & Mirly, 1988 [25] (see text for details).

Glyphosate mean (ppm)	Group 4 Male / Female	Group 5 Male / Female	Group 6 Male / Female
	BLOOD		
Blood plasma	0.129 / 0.127	0.00158 / 0.00114	0.00178 / 0.00152
Red blood cells	0.517 / 0.275	0.00845 / 0.00424	0.00763 / 0.00474
Whole blood	0.328 / 0.166	0.00454 / 0.00269	0.00476 / 0.00288
Bone	30.6 / 19.7	0.552 / 0.313	0.748 / 0.462
Bone marow	4.10 / 12.50	0.0290 / 0.00639	0.0245 / 0.0231
	GLANDS		
Thyroid	1.50 / 1.24	0.000795 / 0.000358	0.00703 / 0.00955
Testes/ovaries	0.363 / 0.572	0.00276 / 0.00326	0.00529 / 0.00813
	ORGANS		
Brain	0.750 / 0.566	0.00705 / 0.00551	0.0144 / 0.0110
Eye	0.655 / 0.590	0.00215 / 0.000298	0.00405 / 0.00337
Heart	0.590 / 0.518	0.00622 / 0.00398	0.00804 / 0.00632
Kidney	1.94 / 1.35	0.0216 / 0.0132	0.0327 / 0.0196
Liver	1.91 / 1.37	0.0298 / 0.0135	0.0407 / 0.0257
Lung	1.54 / 1.13	0.0148 / 0.0120	0.0211 / 0.0167
Spleen	2.61 / 2.98	0.0119 / 0.00727	0.0155 / 0.0130
Uterus	– / 0.618	– / 0.00517	– / 0.00185
	DIGESTIVE SYSTEM		
Stomach	2.38 / 2.36	0.00795 / 0.00367	0.0377 / 0.0239
Small intestine	1.90 / 1.55	0.216 / 0.0183	0.0441 / 0.0257
Colon	11.0 / 9.20	0.0342 / 0.0159	0.0429 / 0.0298
	FAT/MUSCLE		
Abdominal fat	0.418 / 0.457	0.00364 / 0.00324	0.00557 / 0.00576
Testicular/ovarian fat	0.442 / 0.037	0.00495 / 0.00347	0.00721 / 0.00563
Abdominal muscle	0.262 / 0.214	0.00232 / 0.00160	0.00278 / 0.00216
Shoulder muscle	0.419 / 0.423	0.00388 / 0.00667	0.00783 / 0.00590
Nasal mucosa	1.71 / 1.79	0.00485 / 0.0226	0.0316 / 0.0125
Residual carcass	8.78 / 7.74	0.106 / 0.0870	0.157 / 0.101

4.4 Glyphosate metabolites

Howe, Chott & McClanahan [26] identified, characterized and quantified glyphosate and its metabolites after intravenous and oral administration of the radiolabelled compound. They employed several analytical tools, including LSC, strong anion exchange (SAX), cation exchange (CX) and ion pair chromatography (IPC). CX and IPC methods of HPLC were used primarily for the identification of glyphosate and its metabolites contained in urine and faeces. Metabolites of glyphosate found during analysis include the nonbasic compounds aminomethylphosphonic acid (AMPA), methylaminomethylphosphonic acid (MAMPA), N-formylglyphosate, N-acetylglyphosate, N-nitrosoglyphosate and an unknown compound tagged as "Compound #11". Metabolites found in the dosing solutions administered to rats of these experiments would be expected in all glyphosate-based products. CX analysis was used to identify AMPA and MAMPA and IPC was used to identify all other nonbasic glyphosate metabolites. Results are presented in Table 12. Metabolites were also found in the urine and faeces of both male and female rats, as shown in Table 13 for orally dosed groups 4, 5 and 6.

Table 12. Glyphosate and its metabolites: Analysis of dose solutions expressed as % of total. Table adapted from Howe et al. [26].

Dose group	Glyphosate	AMPA	MAMPA	N-acetyl-glyphosate	N-formyl-glyphosate	N-nitroso-glyphosate	Compound #11
1: Oral 10 mg/kg	98.21	0.63	0.26	<0.04	0.49	<0.05	<0.06
3: Intravenous 10 mg/kg	99.14	0.36	0.00	<0.02	0.36	<0.01	0.03
4: Oral 1000 mg/kg	98.88	0.57	0.31	<0.03	0.14	<0.02	0.04
5: Oral 10 mg/kg	99.41	0.17	0.00	<0.03	0.18	<0.03	0.03
6: Preconditioned Oral 10 mg/kg	99.36	0.19	0.07	<0.03	0.21	<0.02	<0.02

Table 13. Glyphosate and its metabolites: Analysis of faeces and urine from male and female rats expressed as % of total. Table adapted from Howe et al. [26].

Dose group	Glyphosate	AMPA (A)	MAMPA (M)	N-Acetyl-glyphosate	N-Formyl-glyphosate	N-Nitroso-glyphosate	Compound #11
4							
Dose solution	98.88	0.57	0.31	<0.03	0.14	<0.02	0.04
Male urine	97.76	1.25 A+M		0.10	0.20	0.09	0.46
Male faeces	98.64	0.82 A+M		<0.03	<0.04	0.13	0.16
Female urine	97.71	1.39 A+M		<0.05	0.25	0.09	0.33
Female faeces	98.68	0.88 A+M		<0.04	<0.04	0.11	0.17
5							
Dose solution	99.41	0.17	0.00	<0.03	0.18	<0.03	0.03
Male urine	99.05	0.32 A+M		<0.05	0.12	0.11	0.31
Male faeces	98.78	0.56 A+M		< 0.06	<0.10	0.21	0.16
Female urine	98.65	0.30 A+M		<0.06	0.25	0.11	0.58
Female faeces	98.23	0.64 A+M		<0.05	<0.09	0.22	0.16
6							
Dose solution	99.36	0.19	0.07	<0.03	0.21	<0.02	<0.02
Male urine	99.24	0.29 A+M		<0.05	<0.11	0.08	0.18
Male faeces	98.31	0.90 A+M		<0.06	<0.10	0.24	0.17
Female urine	98.84	0.26 A+M		<0.04	0.12	0.15	0.51
Female faeces	98.27	0.93 A+M		<0.05	<0.10	0.22	0.23

In vivo metabolization of glyphosate to AMPA was found in the excreta in quantities ≤ 0.4%. The bone was the site of highest bioaccumulation and it retained 0.02 to 0.05% of the oral dose and 1% of the intravenous dose. Repetitive dosing of group 6 animals did not significantly change the metabolization or excretion of glyphosate. Of all of the nonbasic compounds found during analysis of excreta, AMPA followed by N-nitrosoglyphosate were most prevalent. Total N-nitrosoglyphosate levels found in the animals ranged between 0.06–0.20% of the given dose. Faecal samples contained 0.10–0.32% and urine 0.06–0.15% of N-nitrosoglyphosate. Stability studies

revealed that the majority of the N-nitrosoglyphosate found in the faeces was not completely due to presence of the compound as a contaminant of glyphosate, nor was it due to animal metabolism, but rather was due to the chemical reaction of glyphosate with nitrites contained in the excreta. Glyphosate readily reacts with oxides of nitrogen (e.g., NO_2) to form the metabolite N-nitrosoglyphosate. This engenders concern because N-nitroso compounds are carcinogens. Nitrous acid occurring in sweat excreta of the skin could be problematic in the presence of glyphosate and may be responsible for the rise of some skin cancers. N-nitrosoglyphosate, the product of chemical reaction between glyphosate residues and nitrites in the colon, may in fact be a causal agent in the alarming increase in colorectal cancer. We discuss N-nitrosoglyphosate in §8.

Colvin, Moran & Miller [27] evaluated the metabolism of ^{14}C-AMPA in male Wistar rats. A 6.7 mg/kg dose of radiolabelled AMPA was administered orally, of which 20% was found unchanged in the urine of the animals and 74% in the faeces. Recovery from excreta totalled 94% of the dose. In another study, Sutherland [28] fed Sprague Dawley rats a single radiolabelled dose of N-nitrosglyphosate and successfully quantified the metabolite in the urine and faeces. Male and female animals received 3.6 mg/kg and 4.7 mg/kg, excreting 2.8% (faeces) 88.7% (urine) and 10.7% (faeces), 80.8% (urine) respectively. Both male and female rats retained 8.5% of the N-nitrosoglyphosate dose, while 90.5% was eliminated in excreta.

5. THE ISSUE OF CONTROL RATS' DIET

"Historical control data" show that 13–71% of the lab animals used to conduct toxicity tests on various chemicals would spontaneously present with mammary tumours, and 26–93% develop pituitary tumours. Their kidney function is also frequently impaired. A recent study by Mesnage et al. [29] sought to evaluate whether toxic chemicals present in the feed that is standard fare for these animals might be causative for this surprisingly high background rate of disease. Nine out of 13 samples of commonly used laboratory rat feeds tested positive for glyphosate. Thus, these "spontaneous" disease manifestations may well be due to the toxic chemicals in the feed in the control animals rather than to some underlying genetic defects, and this fact raises serious questions about the validity of any studies based on such exposed animals as a control group.

A 1995 paper by Dixon et al. describes a thorough analysis of the frequencies of various organ pathologies related to cancer and other diseases in "control" animals not subjected to any explicit administration of the toxic chemical under investigation [30]. The paper gave no information on the rats' feed or supplements, which would have been important as a possible confounding factor in the observed pathologies, one of which was acinar cell atrophy, present in the pancreas of 6.9% of the males and 5.0% of the female rats. The authors noted a decrease in size and number of acini and increased amounts of interstitial tissue, suggesting fibrosis, along with increased infiltration of lymphocytes and macrophages. Since this is quite similar to the pathology observed with glyphosate exposure to rats, a possibility is that glyphosate contamination in their feed or water supply contributed to the pathology, perhaps in part by chelating manganese; this transition metal is known to stimulate protein synthesis in acini isolated from both diabetic and normal rats and, in the case of diabetic rats, the effect was shown to be specific to manganese (cobalt, nickel, barium, strontium and magnesium failed to exert the effect) [31].

To test for the hypothesis of glyphosate contamination in rat feed, we used HPLC to test for glyphosate and AMPA levels in three distinct rat chow products, containing corn, soy and wheat middlings, and found significant levels of both chemicals in all products examined. We also tested for choline and folic acid. As shown in Table 14, our laboratory analysis of standard rodent diets found no detectable folic acid. Folic acid (folate) is supplied not only through diet but also, particularly, by commensal bacteria via the shikimate pathway [32]. Therefore, glyphosate evidently disrupts folic acid production both in exposed plant food sources and in the human gut, leading to deficiencies. Folate is a cofactor in many important biologic processes, including remethylation of methionine and single carbon unit donors during DNA biosynthesis. This impacts gene regulation, transcription and genomic repair. Folate deficiency enhances colorectal carcinogenesis, in part through impaired DNA methylation [33]. Folate deficiency has also been implicated in the development of several cancers, including cancer of the colorectum, breast, ovary, pancreas, brain, lung and cervix [34]. Folate deficiency during gestation is linked to neural tube defects such as anencephaly and spina bifida.

A synthetic form of choline, choline chloride, has been added to formulated lab chow diets for decades, as indicated from historical references available from manufacturers such as Purina. A 2010 European patent application describes the addition of choline chloride to glyphosate formulations to act as a bioactivator and to enhance penetration of glyphosate into the cells of the target weed [35]. A study of 47,896 male health professionals in the US found that high choline intake was associated with an increased risk of lethal prostate cancer [36]. Our samples all tested positive for choline (see Table 14).

Table 14. Evidence of glyphosate contamination, and levels of folate and choline, in Purina rat chow products as determined from authors' own analyses.

	Glyphosate /mg kg^{-1}	AMPA /mg kg^{-1}	Folate /mg g^{-1}	Choline /mg g^{-1}
Purina Rat Chow 5002	0.65	0.35	0	4.827
Purina Chow 5K75	0.57	0.27	0	5.328
Purina Chow 5LG3	0.37	0.10	0	5.919

The American Veterinary Medical Foundation notes that "Cancer is the leading cause of death in older pets, accounting for almost half of the deaths of pets over 10 years of age." According to the Morris Animal Foundation, established in 1948, one in four dogs will die of cancer and over 22 000 cats will be diagnosed with aggressive sarcomas. Oral cancer squamous cell carcinomas are now found in cats and lead to the destruction of the jawbone. Mammary tumours, a common cancer found in dogs and cats, are also on the rise. We suspect that glyphosate may be a causal agent related to the rise of pet cancers, and used HPLC to analyse 9 popular brands of dog and cat food. We found significant levels of both glyphosate and AMPA in all pet foods tested (Table 15).

Table 15. Glyphosate and AMPA residues found in various dog food and cat food products, as measured from samples tested by the authors.

	Glyphosate /mg kg^{-1}	AMPA /mg kg^{-1}
Purina Cat Chow Complete	0.102	0.12
Purina Dog Chow Complete	0.098	0.076
Kibbles-n-Bits Chefs Choice Am Grill	0.30	0.24
Friskies Indoor Delights	0.079	0.11
9 Lives Indoor Complete	0.14	0.12
Rachael Ray Zero Grain	0.022	Trace (< 0.02)
Iams Proactive Health	0.065	Trace (< 0.02)
Rachael Ray Nutrish Super Premium	0.14	0.14
Purina Beyond Natural - Simply Nine	0.047	0.031

Clearly, it is imperative that future studies on the potential toxicity of any environmental chemical address the issue of the possible toxicity of chemicals contaminating the diet of the control animals, and/or the potential impact of nutritional imbalances. Feeding the control animals an unhealthy diet leads to an increased risk of cancer in the control group making it much harder to see a signal in the experimental group. Furthermore, since oestrogenic chemicals are often more toxic at extremely low doses than at mid-range doses, it is easy to see why the control group may manifest a significant incidence of cancer.

6. EVIDENCE OF DNA DAMAGE FROM THE RESEARCH LITERATURE

According to the IARC's report [2], while there exists only limited direct evidence of carcinogenicity of glyphosate in humans, strong evidence exists to show that glyphosate can operate through two key features of carcinogens: induction of chromosomal damage and induction of oxidative stress. In this section, we review the evidence that glyphosate can damage DNA, a crucial first step leading to cancer. We examine evidence based on sea urchins, children in Malaysia, in mouse models, both *in vitro* and *in vivo*, in human lymphocytes, and in fish. We conclude with a paragraph on folate deficiency, its probable link to glyphosate exposure, and folate's essential rôle in DNA maintenance.

Cell cycle disruption is a hallmark of tumour cells and human cancers. A study on sea urchins investigated several different glyphosate-based pesticide formulations, and found that all of them disrupted the cell cycle. The sprays used to disseminate pesticides can expose people in the vicinity to 500 to 4000 times higher doses than those needed to induce cell cycle disruption [37].

A study on children living near rice paddy farms in Malaysia revealed DNA strand breaks and chromosome breakage associated with reduced blood cholinesterase levels [38], which were attributed to exposure to organophosphate insecticides. The study did not specify exactly to which pesticides the children were exposed, but glyphosate is a general-purpose herbicide whose use in rice paddies in Sri Lanka led to widespread kidney

failure among young agricultural workers there, ultimately resulting in a ban on glyphosate usage in Sri Lanka [4, 5]. While glyphosate is technically an organophosphonate rather than an organophosphate, a study on the fish *Prochilodus lineatus* has demonstrated that it suppresses cholinesterase in both muscle and brain [39].

Bolognesi et al. [40] studied the genotoxic potential of both glyphosate in isolation and Roundup, in both mouse *in vivo* studies and *in vitro* studies of human lymphocytes. In the mouse studies they found evidence of DNA strand breaks and alkali-labile sites in liver but especially in kidney, as well as in bone marrow. Roundup was found to be more toxic than glyphosate, with damage occuring at lower concentrations. They also demonstrated dose-dependent sister chromatid exchanges in human lymphocytes exposed to glyphosate and to Roundup.

A recent study on a 96-hour Roundup exposure to the economically important tropical fish tambaqui found disturbed gill morphology, inhibited cholinesterase activity in the brain and DNA damage in erythrocytes [41]. They found a sixfold increase in a genetic damage indicator (GDI) in erythrocytes, using the "comet" assay method. Similarly, the comet assay applied to goldfish erythrocytes revealed DNA damage following exposure to glyphosate [42], and studies on exposure of eels to realistic concentrations of Roundup and the principal individual components, glyphosate and the surfactant polyethoxylated amine (POEA) in isolation, confirmed DNA damage in erythrocytes [43, 44].

Folate deficiency mimics radiation in damaging DNA through single- and double-strand breaks as well as oxidative lesions [45]. It is estimated that 10% of the US population is at risk from folate deficiency-induced DNA damage. Cancer of the colorectum in particular is linked to folate deficiency [45, 34], which causes reduced bioavailability of cytosine methylation capacity in DNA, inappropriately activating proto-oncogenes and inducing malignant transformation. Folate is also itself crucial for DNA synthesis and repair. Folate deficiency can also lead to uracil misincorporation into DNA and subsequent chromosome breaks [34]. Folate is an essential B vitamin, but it can be synthesized by gut microbes, particularly *Lactobacillus* and *Bifidobacterium* [46]. Glyphosate is a patented *antimicrobial agent*, and these two species are more vulnerable than others to growth inhibition by glyphosate [47]. Furthermore, folate is derived from chorismate, a product of the shikimate pathway that glyphosate disrupts [48].

7. SUCCINATE DEHYDROGENASE INHIBITION

A study on *Escherichia coli* revealed that glyphosate suppressed three different components of the succinate dehydrogenase (SDH) enzyme, cytochrome b556, the avoprotein subunit and the hydrophobic subunit, reducing their activity three- to fourfold [48]. Roundup cytotoxicity in human cells is mediated in part through inhibition of SDH, a key enzyme in mitochondrial complex II [49–51]. A theoretical study of the mechanism of inhibition suggests that glyphosate binds at the succinate binding site with a higher binding energy than succinate, thus blocking substrate bioavailability [52]. Roundup has also been shown to depress complexes II and III [53].

Both SDH (complex II) and fumarate hydratase (FH) (complex III) are tumour suppressors. Their suppressive mechanism can be understood through the effects of enhanced glycolysis following their inhibition [54]. Mutations in SDH lead to the development of paraganglioma (tumours originating in the ganglia of the sympathetic nervous system), and phaeochromocytoma (neuroendocrine tumours of the adrenal glands), and mutations in FH cause renal cell carcinoma. Neuroblastoma is the most common extracranial solid tumour in infants and young children [55]. An increase in growth rate and invasiveness in neuroblastomas is linked to impaired succinate dehydrogenase function [56].

Succinate and fumarate will accumulate in mitochondria when SDH and/or FH are suppressed, and they leak out into the cytosol. Two newly recognized signalling pathways result in enhanced glycolysis in a "pseudohypoxic response", as well as resistance to apoptotic signals [54]. A characteristic feature of tumour cells is their increased use of glycolysis as a source of energy, even in the presence of available oxygen, a phenomenon referred to as the Warburg effect [57, 58]. Malignant, rapidly growing tumour cells typically have glycolytic rates up to 200 times higher than those of their normal tissues of origin, even when oxygen is plentiful.

8. GLYOXAL, METHYLGLYOXAL AND GLYOXYLATE

In this section, we discuss the potent toxicity of multiple metabolites of fructose that are plausibly present in foods derived from glyphosate-resistant crops, or as a contaminant in glyphosate-based products, or as a breakdown product generated endogenously following glyphosate exposure. These include glyoxylate, glyoxal and methylglyoxal. We show that these molecules are genotoxic and can induce cancer. We surmise that their toxicity is enhanced by glyphosate exposure diminishing bioavailability of vitamin E, an antioxidant.

Vitamin E, a tocopherol, is derived from the shikimate pathway, which glyphosate disrupts [59]. One of the best characterized functions of tocopherols is to protect biological membranes against oxidative stress. Superoxide dismutase (SOD) catalyses the conversion of superoxide anion

(O_2^-), a reactive oxygen species (ROS), to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase can also produce ROS, which leads to proteinuria and haematuria [60]. H_2O_2 induces haem degradation in red blood cells, particularly when glutathione is deficient [61]. ROS causes irreversible DNA impairment, damage to lipid membranes and promotes the toxic carbonyl, malondialdehyde [62, 63]. Excessive lipid peroxidation induced with ingestion of glyphosate residues likely leads to an overload of maternal and foetal antioxidant defence systems following liver damage, as shown in rat studies by Beuret et al. [64].

Glyoxal and methylglyoxal are very potent glycating agents, considerably more reactive than either glucose or fructose [65, 66]. They attack the amine groups in amino acids, peptides and proteins to form advanced glycation end products (AGEs), and they cause carbonyl stress in the presence of oxidizing agents such as O_2^- and H_2O_2 [66]. A study linking AGEs to cancer showed that methylglyoxal–bovine serum albumin (methylglyoxal-BSA) induced significant DNA damage [67]. Cancer incidence is increased in association with chronic renal failure, and this is likely due to the binding of AGEs to receptors for advanced glycation end products (RAGE), leading to increased intracellular formation of ROS [67].

Extremely high levels of methylglyoxal are found in commercial carbonated beverages sweetened with high fructose corn syrup (HFCS), but not in those that are sweetened with artificial sweeteners [68]. Since HFCS is derived from glyphosate-resistant corn, it is conceivable that the methylglyoxal was produced in the plant in response to glyphosate exposure. There is a plausible biological mechanism for this, caused by the accumulation of excessive amounts of phosphoenolpyruvate (PEP) as a consequence of the disruption of the enzyme, 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase, that uses PEP as substrate for the first step in the shikimate pathway [69]. PEP suppresses glycolysis by binding to the active site in the enzyme, triose phosphate isomerase (TPI) [70], outcompeting the natural substrates.

Furthermore, PEP reacts with fructose to initiate its conversion to triose phosphate, also known as glyceraldehyde 3-phosphate (glyceraldehyde 3-p), as illustrated in Fig. 1 [71]. Glyceraldehyde 3-p is highly unstable and it spontaneously breaks down to methylglyoxal [72]. Severe impairment of TPI due to genetic defects leads to sharp increases in methylglyoxal and protein glycation, as well as oxidation and nitrosation damage [73]. Inhibition of glycolysis will increase the residence time of glyceraldehyde 3-p and increase its chances to spontaneously degrade to methylglyoxal. It can be expected that similar problems will occur in gut microbes exposed to glyphosate, as well as human cells, and this may explain the increased levels of methylglyoxal observed in association with diabetes [74].

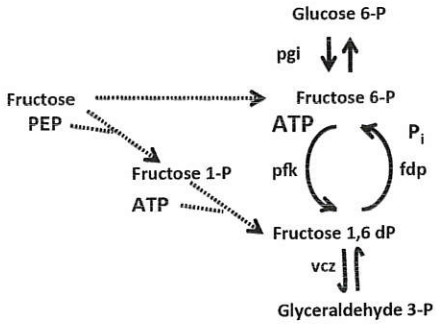

Figure 1. Possible pathways of fructose metabolism in *E. coli*. Genes are pgi, phosphoglucose isomerase; pc, fructose B-phosphate kinase; fdp, fructose diphosphatase; and fda, fructose diphosphate aldolase. PEP, phosphoenolpyruvate. Adapted from Fraenkel, 1968 [71].

A study comparing rats fed a high-fructose compared to a high-glucose diet revealed that those rats fed fructose experienced a significant increase in body weight, liver mass and fat mass compared to the glucose-fed rats [75]. This was accompanied by a reduction in physical activity, although the total number of calories consumed remained equivalent. We suspect that this phenomenon may be largely due to the presence of glyphosate and methylglyoxal contamination in the fructose (which was likely derived from the GMO Roundup-Ready HFCS). A study exposing male Sprague Dawley rats to a high-fructose diet during an interval over a period of four months showed elevated serum levels of methylglyoxal, along with several indicators of diabetes and metabolic syndrome, including expression of RAGE, NF-kB, mediators of the renin angiotensin system and elevated blood pressure [76]. At physiological concentrations, methylglyoxal can modify plasmid DNA and cause mutations and abnormal gene expression [77].

Glyphosate formulations are trade secrets, but a 2006 Monsanto patent proposed using oxalic acid (oxalate) as an additive to increase the toxicity of glyphosate to weeds [78]. Oxalate inhibits pyruvate kinase and this leads to an elevation in PEP along with a reduction in production of lactate and pyruvate. The synthesis of PEP in rat livers exposed to 0.1 mM oxalate more than doubled [79], which likely induces excess exposure to methylglyoxal as discussed above, causing liver stress. The effects of oxalate would be synergistic with the effects of glyphosate inhibition of the shikimate pathway in gut microbes, which can be expected to also increase PEP levels, since PEP is substrate for the enzyme that glyphosate disrupts.

Several anaerobic bacteria, including *Oxalobacter formigenes*, *Eubacterium lentum*, *Enterococcus faecalis* and *Lactobacillus acidophilus* can metabolize oxalate in the gut [80]. However, both oxalate decarboxylase and oxalate oxidase, enzymes involved in oxalate metabolism, depend on manganese as a cofactor [81], and manganese is chelated by glyphosate, making it unavailable to gut microbes [82, 83].

Elevated serum glyoxylate has been found to be an early marker for diabetes risk [84]. The conversion of glyoxylate to oxalate by the enzyme lactate dehydrogenase is inhibited by oxalate [85, 86]. Hence glyoxylate, derived from glyphosate breakdown, can be expected to accumulate in the presence of excess oxalate. Glyoxylate can be derived from glyoxal, and both glyoxal and glyoxylate have been proposed as key reactants in the production of glyphosate, as described in multiple patents from the mid-1980s [87, 88]. Furthermore, glyphosate can itself be metabolized to AMPA and glyoxylate by microbial action along two distinct pathways, via glycine oxidase or via glyphosate oxidoreductase [89]. *In vitro* exposure of hepatocytes to glyoxal showed hepatotoxicity induced by lipid peroxidation, ROS, and collapsed mitochondrial membrane potential [90, 91].

LDH is also known to be involved in tumour metabolism. A Monsanto study conducted by Johnson on rabbits found that extremely high doses of glyphosate (5000 mg/kg) severely downregulated production of LDH, reducing values in both male and female animals, whereas a fivefold lower dose (1000 mg/kg) upregulated LDH similarly in both males and females compared to the experimental control [92]. Glyphosate was administered by dermal absorption to three groups, each of 5 male and 5 female rabbits. Doses of 100, 1000 and 5000 mg/kg were held in place by occlusion for 6 hours/day, five days/week for 21 days. A control group of the same numbers of animals and sex did not receive the compound. Results for the control, low-, mid- and high-dose groups were 250, 169, 291 and 76 for male animals and 189, 149, 258 and 28 for female animals, respectively. Not understanding glyphosate's nonmonotonic dose-response relationship caused Johnson to dismiss this haematological finding. A similar pattern of LDH regulation was recorded by Stout & Ruecker in 1990 in experiments with albino rats [15].

A Monsanto patent application from 1985 describes the invention as follows: "glyphosate and various glyphosate derivatives can be produced with very high selectivity by the reductive alkylation of aminomethylphosphonic acid, its salts or its esters, in an aqueous medium with a carbonyl compound, such as, for example, glyoxal, glyoxylic acid, a glyoxylate salt, or a glyoxylate polyacetal salt or ester" [88]. An earlier US patent application disclosed a similar process whereby aminomethylphosphonic acid is reacted in an aqueous medium with glyoxal in the presence of sulfur dioxide to produce glyphosate. Methylglyoxal is cytotoxic, and it has been shown to arrest growth and react with nucleotides, increasing the incidence of sister chromatid exchanges, a step towards tumorigenesis [93].

Methylglyoxal also decreases protein thiols, especially glutathione, an essential antioxidant. In *in vitro* studies, glyphosate has also been shown to reduce glutathione levels in mammalian cells, possibly mediated through methylglyoxal [94]. Methylglyoxal induces DNA mutations mainly at G:C base pairs, and it severely inhibits DNA replication by inducing cross-links between DNA and DNA polymerase [95]. The mutagenicity of methylglyoxal is suppressed by sulfur-containing molecules, such as sulfite, cysteine and glutathione [96]. Glyphosate has been shown to deplete methionine levels by 50% to 65% in a glyphosate-sensitive carrot plant line [97]. Methionine is an essential sulfur-containing amino acid crucial for maintaining levels of cysteine, glutathione and sulfate. Most bacteria possess biosynthetic pathways for methionine [98], and it is possible that glyphosate disrupts their ability to supply this critical nutrient to the host.

Glyoxalase is a key enzyme in the pathway that detoxifies methylglyoxal. Mouse studies have demonstrated that its overexpression can reduce AGE production and oxidative damage associated with hyperglycaemia [99], thus demonstrating a direct link between methylglyoxal and these pathologies. Glyoxalase is upregulated in association with rapid cell proliferation [100] and also in association with some cancers, including gastric cancer [101] and prostate cancer [102] (gastric cancer is the second highest cause of cancer-related mortality worldwide [103]). Overexpression of glyoxalase I is associated with increased gastric wall invasion and lymph node metastasis [101]. Glyphosate exposure has been shown experimentally to induce increased expression of glyoxalase activity in *Arachis hypogaea* (groundnut), which was engineered to be glyphosate-tolerant [100]. In addition, the observed upregulation of redox-regulated kinases, phosphatases and transcription factors shows the importance of redox couples to reorganize growth and metabolic needs under stress conditions, such as exposure to glyphosate. Mitogen-activated protein kinase (MAPK) phosphatases (MKPs) play an important rôle in the development of cancer in humans [104].

9. N-NITROSOGLYPHOSATE AND N-NITROSOSARCOSINE

As was shown by Monsanto's own studies [26], glyphosate readily reacts with nitrogen oxides to form N-nitrosoglyphosate (NNG), which is of great concern due